CASQ2-knockout animals (Knollmann et al., 2006. *J Clin Invest* 116:2510). Lack of both CASQ isoforms was confirmed by western blot. The double-null mice are viable and breed normally, however the rate of spontaneous mortality of male animals is higher than CASQ1-null animals. Whereas the overall phenotype of mice is similar to that of CASQ1-null mice, significant differences are found in Soleus. From the structural point of view, in Soleus muscle we find many fibers (about 30%) with severe structural damage that were not found in CASQ1-null animals. Functional studies indicate significant prolongation in twitch time parameters, increased twitch tension and impaired tension generation during prolonged tetani both in EDL and Soleus, likely related to abnormal calcium release kinetics. These findings suggest that: a) expression of CASQ2 is essential for the maintenance of a subpopulation of Soleus fibers; and b) lack of both CASQ1 and 2 exacerbates the overall phenotype of CASQ1-null mice.

2821-Pos

Refractoriness of Sarcoplasmic Reticulum Calcium Release in Cardiac Muscle Due to Calsequestrin

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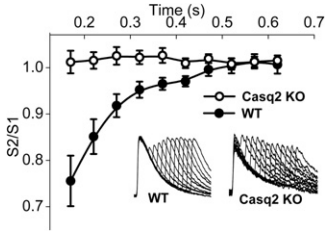
In cardiac excitation-contraction coupling, L-type Ca^{2+} current (I_{Ca}) triggers Ca^{2+} release from the sarcoplasmic reticulum (SR) via ryanodine receptor (RyR) Ca^{2+} release channels. It is unclear why SR Ca^{2+} release cannot be elicited by premature stimuli, even though I_{Ca} is fully recovered. Here, we use calsequestrin null mice (Casq2 KO) and wild-type littermates (WT) to test the hypothesis that calsequestrin (Casq2) determines refractoriness of SR Ca^{2+} release. Ca^{2+} release refractoriness was measured in voltage-clamped myocytes dialyzed with Fluo-4 by applying premature extrastimuli (S2) at successively shorter S1-S2 coupling intervals following a 1 Hz train (S1 stimuli). To maintain constant trigger, Ca^{2+} release was activated with I_{Ca} tail currents that elicited maximal Ca^{2+} release during the S1 train. WT S2 Ca^{2+}release was significantly depressed with short coupling interval whereas Casq2 KO cardiomyocytes exhibit no refractoriness of Ca^{2+} release (Figure, n=11 WT, 12 KO, p = 0.01). At the same time, I_{Ca} current density, SR Ca^{2+} content, and steady-state Ca^{2+} transients (S1) were not significantly different from WT-myocytes. We conclude that calsequestrin is a critical determinant of SR Ca^{2+} release refractoriness in cardiac muscle (Supported by NIH-R01HL71670, R01HL88635).



2822-Pos

Effect of Triadin on Retrograde and Orthograde Signaling between RyR1 and DHPR in Cultured Myotubes

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Using pan triadin-null mice we previously showed that triadins ablation did not disrupt EC-coupling in muscle cells. However, calcium imaging studies in cultured myotubes did reveal that triadin-null myotubes had slightly smaller depolarization-induced Ca^{2+} transients than Wt cells. Here, using whole-cell voltage clamp, we analyze the effect of triadin ablation in skeletal EC-coupling by characterizing the retrograde and orthograde signaling between RyR1 and DHPR of triadin-null myotubes. Calcium currents elicited by 200ms depolarization steps in Wt and triadin-null cells showed slow kinetics of activation and peak current at approximately +30 mV. Although, the overall voltage dependence was preserved between Wt and triadin-null cells a leftward shift in the I/V curve was observed in triadin-null cells ($V_{1/2}$, 22.3 ± 0.8 mV in Wt vs 16.6 ± 1.1 mV in triadin-null cells, $p<0.05$). In addition, kinetic analysis of the DHPR Ca^{2+} current shows that the activation time constant of the slow component (τ_{slow}) was slightly decreased from 37 ± 2.4 ms in Wt to 26 ± 2.6 ms ($p<0.05$) in triadin-null cells.

The voltage-evoked Ca^{2+} transient, on the other hand, showed a small but significant reduction of the peak fluorescence amplitude of triadin-null cells ($\Delta F/F_{max}$, 0.72 ± 0.2 in Wt vs 0.61 ± 0.1 in triadin-null) with no differences in voltage dependence (V_m, -7.2 ± 1.1 mV in Wt vs -10.1 ± 1.9 mV in null cells). Our results suggest that the absence of triadin expression preserves the orthograde and retrograde signaling between DHPR and RyR1 nearly intact and that the effect of triadin ablation on $\Delta F/F_{max}$ would be secondary to the dysregulation of calcium homeostasis observed in triadin-null cells. These data give further support to the idea that skeletal triadins do not play a direct role in skeletal EC-coupling.

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2823-Pos

Altering Skeletal Muscle EC Coupling by Ablating the Sarcoplasmic Reticulum Protein JP45 Affects Both Metabolism and Muscle Performance in Old Mice

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JP45, a sarcoplasmic reticulum protein, appears to be mainly expressed in skeletal muscle. In mice, its expression is down-regulated during aging, and it interacts with the α1.1 subunit of the dihydropyridine receptor (Cav1.1) and calsequestrin, two key components of the excitation-contraction (EC) coupling machinery.

We examined 12- and 18-month-old JP45 knock-out mice and compared them with age-matched, wild-type littermates. The JP45 KO mice exhibit a phenotype consistent with impaired skeletal muscle EC coupling, confirming our previous results in young JP45 KO mice. Spontaneous motor activity assessed with a running wheel revealed that the older JP45 KO group runs less and much more slowly than age-matched WT and young JP45 KO mice. *In vitro* muscle contractile property analysis showed lower twitch and tetanic absolute and specific force, evident mostly in the EDL of aged JP45 KO mice compared to age-matched WT, which correlates with type-II fiber atrophy. Cav1.1 expression and SR Ca^{2+} release in voltage-clamped flexor digitorum brevis muscle fibers of aged JP45 KO mice were reduced compared to age-matched WT. Additionally, aged JP45 KO mice exhibited decreased food intake and body weight.

Our results show that JP45 plays a role in EC coupling and regulation of body metabolism. *Supported by NIH/NIA, Japanese Science Foundation, M.U.R.S.T., A.F.M., and Swiss Muscle Foundation.*

2824-Pos

Characterization of Calumenin-RyR2 Interaction in Murine Heart

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Calumenin is a multiple EF-hand Ca^{2+}-binding protein localized in the sarcoplasmic reticulum (SR). In our recent study, we showed that calumenin-knockdown (KD) of HL-1 cells led to enhanced Ca^{2+} release and Ca^{2+} uptake in the SR (*Sahoo et. al. J. Biol. Chem.*, 2009). To elucidate the underlying mechanisms responsible for the enhanced Ca^{2+} release from the SR in calumenin-KD samples, the possible interaction between calumenin and RyR2 was examined by various methods. GST pull-down assay showed a direct interaction between calumenin and RyR2. We have further found that the middle region of calumenin (aa 132-222) interacts with RyR2. GST pull-down assay also shows that RyR2 intra luminal loop-I region (aa 4519-4576) is the binding site for calumenin. Immunofluorescence study shows that RyR2 and calumenin are co-localized in the junctional region of SR in rat ventricular cardiomyocytes. The detailed amino acid residues involved in the interaction between calumenin and RyR2 are currently under investigation. (This work was supported by the Korean Ministry of Science and Technology grant, Systems Biology Research Grant, M1050301001-6N0301-0110, and the 2009 GIST Systems Biology Infrastructure Establishment Grant).

2825-Pos

Progressive Triad-Mitochondria Un-Coupling in Aging

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An impairment of the mechanisms controlling the release of calcium from internal stores (excitation-contraction (EC) coupling) has been proposed to contribute to the age-related decline of muscle performance that accompanies aging (EC un-coupling theory). EC coupling in muscle fibers occurs at specialized intracellular junctions called calcium release units, or triads, which are specifically placed at sarcomere's I-A band transition. In recent publications we have shown that: a) in human muscle, the frequency of triads decreases significantly with age (Boncompagni et al., 2006; J Gerontol 61:995); and b) in mice, triads are tethered to mitochondria placed at the I band (Boncompagni et al., 2009; MBC 20:1059). Here we have studied the frequency, sarcomeric-localization, ultrastructure, and coupling of triads/mitochondria in EDL from male WT mice using transmission electron microscopy (TEM).

Preliminary results indicates that the number of triads/100µm2 of longitudinal section in aging mice (n=4, 25-35 months of age) decreases compared to the adult mice (n=5, 3-12 months of age): 92 ± 9 vs. 79 ± 8. In addition, the percentage of abnormally positioned triads (longitudinal and/or oblique) increases. On the other hand, the total volume of mitochondria does not change significantly with age. However, the number of mitochondria-profiles/100µm2 of

longitudinal section is reduced (54 ± 7 vs. 43 ± 6), suggesting a remodelling/fusion of these organelles. Finally, we have assessed the positioning of mitochondria in respect to myofibrils and triads: a) the number of mitochondria at the A band (misplaced) slightly increases with age (9% vs 3%), whereas the number of triads-mitochondria couples is significantly reduced: 39 ± 5 vs. 26 ± 4. Our observations indicates: a) a age-related partial disarrangement and spatial re-organization of EC coupling/mitochondrial apparatuses; and b) a decreased percentage of mitochondria functionally tethered to calcium release sites. This could in part explain the decline of muscle performance associated to increasing age.

2826-Pos

Knockdown of TRIC-B from *tric-a*$^{-/-}$mice Alters Intracellular Ca^{2+} Signaling in Skeletal and Cardiac Muscles

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Trimeric intracellular cation (TRIC) channel subtypes are present in the endo/sarcoplasmic reticulum (SR) and nuclear membranes of muscle cells and other tissues. Knockout mice lacking both TRIC-A and TRIC-B channels suffer lethal embryonic cardiac failure due to dysfunctional intracellular Ca^{2+} signaling in the mutant cardiomyocytes (Yazawa et al., Nature 448, 78-82). The lethality associated with double knockout of *tric-a* and *tric-b* prevents physiological assessment of TRIC channels in adult tissues. Here we took advantage of the viable *tric-a*$^{-/-}$ mice and employed RNAi-mediated knockdown of *tric-b*, in order to examine the physiological function of TRIC channels in adult muscle cells. We used electroporation-mediated delivery of shRNA against *tric-b* into the flexor digitorum brevis (FDB) muscles of living *tric-a*$^{-/-}$ mice. Individual FDB fibers with knockdown of TRIC-B were used to examine the Ca^{2+} sparks properties in response to osmotic stress, and voltage-induced Ca^{2+} release under voltage clamp. Compared with the *tric-a*$^{-/-}$ muscle treated with control shRNA, acute knockdown of TRIC-B leads to significant reduction of the amplitude of Ca^{2+} sparks accompanied with prolongation of the duration of Ca^{2+} sparks. In neonatal cardiomyocytes isolated from the *tric-a*$^{-/-}$ mice, knockdown of TRIC-B led to significant perturbation of Ca^{2+} signaling from the SR, evidenced by irregular intracellular Ca^{2+} signaling and reduced frequency of spontaneous Ca^{2+} oscillations. These results indicate that disruption of TRIC function can alter intracellular Ca^{2+} signaling in skeletal and cardiac muscles and this may underlie an increased susceptibility of these tissues to various physiological stresses.

2827-Pos

Local Ca^{2+} Releases Enable Rapid Heart Rates in Developing Cardiomyocytes

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Homogeneous intracellular Ca^{2+} release repeated with high frequency is the basis of the rhythmic contractions of cardiac myocytes. In adult ventricular myocytes, the t-tubular system enables transient homogeneous Ca^{2+} signals. Interestingly, the developing cardiomyocytes do not have t-tubuli and Ca^{2+} signal propagation in the cytosol is based on the relatively slow diffusion of Ca^{2+} ions. This is likely to result in spatiotemporal heterogeneity of Ca^{2+}, which limits the maximal frequency of the Ca^{2+} signals. We observed that intracellular Ca^{2+} signals of 12.5 days old mouse embryonic ventricular myocytes are more homogeneous than expected if the Ca^{2+} signals would propagate by pure diffusion. To study the propagation more accurately, we injected a small amount of Ca^{2+} to a single point in the cytosol via patch-clamp pipette while performing the line-scan imaging of the intracellular Ca^{2+}. With this method we found that inhibition of the sarcoplasmic reticulum (SR) Ca^{2+} release channels results in 3-fold slowing of Ca^{2+} signal propagation (control: 10.1 ± 2.7 ms/μm vs. ryanodine (50 μM): 33.6 ± 9.2 ms/μm, $P < 0.05$). This suggested that the propagation of Ca^{2+} signals is amplified with local SR Ca^{2+} releases. Immunolabeling of SR Ca^{2+} release and uptake proteins revealed a regular structure throughout the cytosol at ~2 μm intervals. These extensions of SR were equally functional in all parts of the cytosol. To further study the role of these local Ca^{2+} release sites in developing cardiomyocytes, we implemented a model of them into the previously published mathematical model of an embryonic cardiomyocyte. The computer simulations showed that the local Ca^{2+} releases are prerequisite for synchronizing the global intracellular Ca^{2+} releases upon electrical excitation and maintaining the capability of developing cardiomyocytes to generate spontaneous pacemaking at a sufficiently high frequency.

2828-Pos

Ca^{2+} Transients and Myosin Heavy Chain (MHC) Composition in Murine Enzymatically Dissociated Fibers

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Single and tetanic Ca^{2+} transients reported with MagFluo4-AM were obtained together with MHC electrophoretic patterns in enzymatically dissociated fibres from adult mice soleus and extensor digitorum longus (EDL) muscles. Kinetics of transient rise (Ca^{2+} release) and decay (Ca^{2+} clearance) of both twitch and tetanic responses showed a continuum from the slowest records obtained in fibers type I, to the fastest obtained in fibers IIX/D and IIB. Fibers IIA were fast regarding Ca^{2+} release but slow regarding Ca^{2+} clearance. Single transients decay was described by a double exponential function with time constants (τ_1 and τ_2, ms) of 3.2 and 49.5 in soleus (types I and IIA, n=23) and 1.6 and 10.5 in EDL fibres (types IIX/D and IIB, n=16). These time constants were associated with components A1 and A2 (%) of 28.1 and 71.9 for soleus, and 35.8 and 64.2 for EDL. For all fiber types, after few repetitive stimuli at 100 Hz there was a big change of decay kinetics compared to single transients and then mild changes were seen in records lasting from 50 to 350 ms. In EDL tetanic transients, the fast component A1 almost disappeared, leaving the A2 and a much slower third one (A3) with τ_2 and τ_3 of 14.6 and 1259.7 (n=6). In soleus the A1 disappeared, while A2 increased with a τ_2 of 74.6 (n=5). Preliminary experiments using CPA (1-2 μM) and FCCP (2-4 μM) have shed some light into the mechanisms involved in relaxation of tetanic transients in different fiber types. In conclusion, we show for the first time the diversity of Ca^{2+} transients in the whole spectrum of fibre types and correlate it with the structural and biochemical diversity of mammalian skeletal muscle fibres. (FONACIT G-2001000637).

2829-Pos

Effects of γ-Ketoaldehydes on Ca^{2+} Cuttrent Induced SR Ca^{2+} Release in Ventricular Myocytes

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• Oxidation increases RyR2 channel activity, enhances cardiac SR Ca^{2+} release and causes spontaneous SR Ca^{2+} waves. Isoprostanes have become a recognized marker of oxidative stress in rodents and humans. γ-ketoaldehydes(γ-KAs) are the most reactive product of the isoprostane pathway. Recently, we found that lipophilic pyridoxamine analogues, salicylamine(SA) scavenge γ-KAs and thereby prevent formation of γ-KA protein adducts in response to oxidative stress. We hypothesized that γ-KAs are potential mediators of oxidant-induced RyR2 channel dysfunction and spontaneous SR Ca^{2+} waves, and that SA would prevent oxidant-induced spontaneous SR Ca^{2+} waves(SCW) in the ventricular myocytes.

• We compared the effect of γ-KAs(1uM) or H_2O_2(10uM) and the effect of SA on Ca-current induced Ca release(CICR) in murine ventricular myocytes loaded with Fura-2AM or Fluo-4. All data are expressed relative to vehicle (Mean ± SEM, n=15-50 per group).

• Acute exposure(3 min) to γ-KAs(1 uM) or H_2O_2(10 uM) increased the amplitude of Ca^{2+} transients, and the fraction of Ca^{2+} released from the SR(γ-KAs130 ± 10%*, $H_2O_2$120 ± 10%, *p<0.05) during each beat. Furthermore, the rate of SCW was significantly increased(γ-KAs 42%*, $H_2O_2$33%*, *p<0.05) and SR Ca^{2+} content was reduced. In voltage-clamped myocytes, dialysis with γ-KAs enhanced Ca^{2+} release without changing L-type Ca^{2+} current, demonstrating that the effect of γ-KAs is the result of RyR2 modification. However, after chronic exposure(30 min) to γ-KAs(1 uM) or H_2O_2(10 uM), Ca^{2+} transients(γ-KAs 0.53 ± 0.1*, H_2O_2 0.7 ± 0.1*, *p<0.05) and SR Ca^{2+} contents decreased, and SCW remained elevated. Pre-treatment(3 days) of salicylamine reduced H_2O_2-induced spontaneous Ca^{2+} waves(SCWs/sec, $H_2O_2$1.2 ± 0.3*, SA-$H_2O_2$0.4 ± 0.2*, *p<0.05) preserved with SR Ca^{2+} content in ventricular myocytes.

• We found that H_2O_2 and γ-KAs have analogous biphasic effects on SR Ca^{2+} release in ventricular myocytes. The protective effect of γ-KA scavengers suggests that γ-KAs are possible mediators of oxidant-induced RyR2 channel dysfunction.

2830-Pos

CamkII Phosphorylation of RyRs: a Mechanistic Mathematical Model

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